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ON EXPERIMENTAL AMYLOID DEGENERATION WITH SPECIAL REFERENCE TO THE EFFECT OF SUPER-NORMAL TEMPERATURE ON ITS DEVELOPMENT

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FOREWORD

The object of this study was primarily to continue the clinical work on amyloid degeneration which one of us carried out in a series of cases of chronic empyema of the pleura. [1] For this purpose we first tried to ascertain the degree to which temperature affects the development of amyloid degeneration in amyloid conditions produced experimentally. Of course results obtained in experiments on animals are not directly comparable with diseased conditions in human beings. Just as human beings are differently predisposed to diseases and especially to amyloid degeneration, predisposition varies considerably also in animals and even in the same animal. The results obtained in experimental investigations may, with certain provisos, be of advantage to clinical research.

In the course of our work we noticed in several laboratory animals reactions which seemed to bear out Selye's theory on the general-adaptation-syndrome. Although it was not originally one of our chief aims, we have devoted some attention to this reaction of the organism.

We would like to thank Professor P. E. A. Nylander, M. D., Head of the First Surgical Clinic of Helsinki University, who encouraged us to undertake this work and gave us valuable advice and instruction. To the *Wihuri Research Institute* we owe our thanks for allowing us to carry out our work in its premises. The laboratory animals were provided by the Institute. We are, morever, grateful to the Head of the Institute, Dr. Pentti I. Halonen, for his sympathetic interest in our work.

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[1] Peräsalo, O.: Studies of Metabolism in Cases of Chronic Empyema of the Pleura with Reference to Amyloid Degeneration. — Ann. Chir. Fenn. 1948: Vol. *37:* Suppl. 2.

We are indebted to Professor U. Uotila, M. D., Head of the University Institute of Forensic Medicine, Helsinki, for checking the histological part of the work and helping us with his advice. We have to thank Dr. V. Ritama for helping us particularly in the choice of staining methods. Our thanks are also due to Miss Maj-Lis Åberg, laboratory superintendent at the Maria Hospital, who assisted in the analysis of the bone-marrow preparations.

I

GENERAL CONSIDERATIONS; OBJECT OF THE PRESENT STUDY

Even nowadays there are two sides to the amyloid problem: first, the question of its chemical construction and the significance of the colour reactions characteristic of it, and, second, the actual aetiology of the disease. As regards the chemical construction of amyloid, it is known to be a large molecule protein belonging primarily to the globulins.

There are several theories about the formation of amyloid in the organism. MEYER and WULF, who carried out research during the 1914—1918 war, were of the opinion that food played an important part in its formation. They assumed that an increased disintegration of protein occurs in connection with a disturbance in sulphur metabolism. LETTERER (1926, 1934) and LOESCHKE (1927) published a more widely accepted theory according to which the precipitation of protein was the consequency of an antigen-antibody reaction. In typical amyloidosis the precipitation would occur in connection with an antigen surplus, instead of during the formation of antibodies. Tissue or leukocyte albumin would then very likely act as an antigen. According to APITZ (1940) typical amyloid degeneration would be caused by functional disturbances in the cellular system which forms the protein of the body, as the result of the increased activity caused in the cells by infectious diseases.

It has been proved in laboratory experiments that amyloid precipitation occurs in the test animal as a result of various intoxications and that when the intoxication disappears the amyloid is resorbed. It is especially interesting to note that amyloid does not

in these cases develop only as a result of pus bacteria but also as a result of purely chemical substances, which are not biological products of micro-organisms. Oil of turpentine and protein are among such substances. Birch-Hirschfeld (1899) was the first to succeed in producing amyloid experimentally, by injecting pus into rabbits subcutaneously. Novak (1898) produced amyloid by means of oil of turpentine and Schepilewsky (1899) by lab ferment. Kuczynski (1923) fed the test animal with plenty of casein, a food rich in protein, or injected it subcutaneously. By this last procedure amyloid developed in 10 to 30 days. When it was given per os the results were less evident. Along with a reduction in the supply of protein the amyloid also disappeared. Smetana (1925) and Letterer (1925), among others, came to the some conclusions. Jaffe (1926) studied the effect of fats, especially cholesterol, in experimental amyloidosis. He noted that when he gave the mice plenty of food rich in cholesterol they needed double the amount of nutrosis compared with animals who had been on food poor in cholesterol, before amyloid developed. Niosi (1937) brought about amyloid degeneration in mice more easily after the removal of the spleen. It is also known that amyloid often occurs in so called serum horses as a consequence of repeated injections of diphtheria toxin (Hjärre, 1945).

It is common to all these experiments that they cause an absolute increase in the globulin content of the blood, as Eklund and Reimann (1936) have shown in similar experiments on rabbits. *Chronic hyperglobulinaemia thus seems to be an important factor in the development of amyloidosis. When the protein surplus from the blood is stored in the tissue, amyloid forms and the hyperglobulinaemia decreases* (Beattie and Dickson, 1943). Björneboe and Gormsen (1943) showed in their experiments that in immunized rats the increase in serum globulin occurred contemporaneously with the formation of antibodies. They explained that the increase in globulin can be considered a result of the production of the antibodies. According to recent literature hyperglobulinaemia is characterized by an increase in plasma cells and cells belonging to the reticulo-endothelial system, which leads us to suppose that the formation of globulin is connected with these cells (Fleischhacker 1940; Apitz 1940, Ranström, 1946 etc.). Amyloid and hyperglobulinaemia are biological abnormalities occurring especially in connec

tion with infectious diseases. Hyperglobulinaemia is a general reaction of the organism and arises from the most various causes, and its biological significance is still unknown.

There are different theories on the *formation of blood protein* in the organism. According to the latest views the reticuloendothelial system is of primary importance. Aschoff and Landau state that the reticulo-endothelial system consists of a special group of cells which are distinguishable by their reaction to certain dyes. They included in this group the endothelium of the blood and lymph vessels etc., fibrocytes, the reticular cells of the spleen pulp, Kupfer's star cells, the endothelium of the capillaries of the bone marrow, as well as histiocytes and monocytes. Also in the cortex of the adrenals and the hypophysis there are types of cells belonging to this group.

The reticulo-endothelial system of the bone marrow consists of cells among which Rohr (1940) distinguishes 5 types. These are: small and large lymphoid reticular cells, phagocytizing reticular cells, fat cells of the bone marrow, and endothelial cells. Among these reticulo-endothelial cells Rohr also includes plasma cells, which comprise about 1 per cent of the marrow cells with a nucleus. Some writers treat these cells, which bear a close resemblance to lymphocytes, as a separate group (Fleischhacker). It is characteristic of the cells belonging to the reticulo-endothelial system of the bone marrow that they are capable of absorbing and storing various substances. Plasma cells, on the other hand, have not this capacity, as was pointed out by Klima, Markoff, Rohr and Naegeli. They seem to be capable of discharging different substances and are of especial significance in the secretion of plasma protein, in maintaining the protein content of blood.

Some clinical investigations bring out the importance of bone marrow in the formation of protein. It has been found that in severe lesions of the bone marrow the fibrinogen content of blood shows a marked decrease. E.g. Jürgens and Trautwein (1930) published a case of prostate carcinoma with extensive bone marrow metastasis. This patient suffered from very marked fibrinopaenia and his blood had completely lost its power of coagulation. Fleishhacker, among others, recorded a similar case, characterized by abundant subcutaneous haemorrhage.

From this survey of literature we note that no attention has been paid in experimental investigations dealing with amyloid to the possibility of temperature promoting the development of amyloid. In the present study we have concentrated on this point. Yet the way in which amyloid was produced for the purposes of this study differs from the physiological production of amyloid in human beings in association with certain diseases. It might therefore be assumed that in research made on test animals certain reactions occur whose primary purpose is to raise the power of resistance of the organism to a definite irritant. To a limited degree we have, in fact, been able to notice reactions pointing to the so-called general-adaptation-syndrome.

The questions we have endeavoured to answer in the present study are the following:

1. *Does super-normal temperature in the test animal contribute to the development of amyloid?*

2. *What kind of changes take place during the development of amyloid*

   a. *in the ratio of the plasma proteins*
   b. *in the amount of the leukocytes of the blood*
   c. *in the bone marrow. Above all, can any increase in the number of cells belonging to the reticulo-endothelial system be noted?*

3. *To what extent can reactions observed in connection with these experimental amyloid investigations be said to point to the general-adaptation-syndrome?*

II

MATERIAL AND METHODS

White mice and rats were used as test animals. Their number was 68 in all, 59 mice and 9 rats, besides which we used 10 mice and 5 rats as controls. Mice were chosen for the purpose primarily because amyloid can be produced in them in a comparatively short time. In rats, on the contrary, a much longer time may elapse before any amyloid formation is discovered.

The substance used for producing amyloid was a 5 per cent sodium caseinate emulsion, of which 1 cc was injected into the test animals subcutaneously every other day. The test animals were divided into two groups, and the formation of amyloid was watched in two parallel experiments. One group of the mice was kept in a terrarium with a temperature kept at 39°—41° C by means of two shaded 60 Watt electric bulbs, and the other group at ordinary room temperature. If the temperature was 42° C or above, the mice could not stand it but often died the next day. Exactly the same kind of food was given to both groups, consisting of vegetables, oats, and bread.

Sodium caseinate emulsion was injected into both groups in exactly similar amounts and on the same day. The number of injections varied from 2 to 15. After about one week from the beginning of the injections the first investigations were made on the first two mice. The samples were taken in the following way:

The blood vessels of the neck were first prepared under aether anaesthesia. Blood was drawn from them into a test tube containing two drops of heparin, for estimating the albumin and globulin content of the plasma. The liver, the spleen and a kidney were then taken with a view to ascertaining, histologically, possible amyloid. For an investigation of the bone marrow cells a specimen was taken from the femoral bone marrow. All these investiga-

tions were also made on the normal 10 mice and rats used as control material. The quantitative determination of the plasma proteins was carried out by the biuret method.

The preparations taken for the histological examination were fixed in 10 per cent formalin and stained both by the van Gieson and Kongo red methods and sometimes by BAUER's method. In doubtful cases iodine and crystalline violet stains was also used. We first used the sternum for investigating the bone marrow cells, but because of the small amount of the marrow it was difficult to obtain satisfactory slide preparations. We therefore made use of the femoral bone. The preparations obtained from it were, however, too thick, which made analysis difficult. By adding a drop of 30°—35° saline into the marrow pulp on the slide it was possible to improve the preparations.

III

OWN INVESTIGATIONS

EXPERIMENTS TO PRODUCE A RISE IN TEMPERATURE

To produce a rise in temperature we first experimented with »Pyrisan» a suspension made by the Orion Pharmaceutical Manufacturing Co. Ltd., of various strains of Bact. fae. alcaligenes, and sulphurated sulphurol (sulphur praec. 0.005, benzyl carbinol. 0.02, o., arachid. ad. 1 ml.). These substances were injected subcutaneously into the mice in different dilutions. We tried to keep the external conditions as similar as possible for the test animals.

The normal temperatures of 7 mice were first determined by measuring the temperature per rectum several times a day on successive days. 36.1° C was established as the average normal temperature of the mice. Even the injection of comparatively large amounts of Pyrisan produced no in rise temperature or only a very small one. The results of the investigations made with »Pyrisan» appear in Table 1.

TABLE 1

NORMAL TEMPERATURES OF MICE AND EXPERIMENTS TO PRODUCE A RISE IN TEMPERATURE WITH PYRISAN (HR = TIME IN HOURS AFTER THE INJECTION)

Time	No.	Pyrisan Units	Temperature C°						
			Norm	½ hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
18/12 47	C_1	1.25	36.3		35.5	35.8			
7/1 48	»	12.5	35.8		36.0	36.0			
17/1 48	»	125	34.8	35.2	35.3	35.6	37.4		37.7
8/1 48	C_2	25	35.4			36.8	36.6		36.4
8/1 48	C_3	37.5	36.0			35.0	35.0		35.0
13/1 48	C_4	125	35.8		37.9	34.7	34.6		34.6
14/1 48	C_4	625	34.4	34.7	34.8				
17/1 48	C_4	1250	35.6	35.2					
17/1 48	C_5	250	37.0		37.6	35.0	35.0	35.1	35.0
17/1 48	C_6	250	37.4	36.3	35.7				
17/1 48	C_7	625	36.0	35.6	34.6				

The experiments showed that no rise in temperature could be produced in mice with Pyrisan. The results obtained with sulphurol were similar. We therefore could not use these substances for causing fever. Milk injections were not found suitable either, since milk, which contains protein, might, in connection with casein, promote the development of amyloid. We now tried to bring about a condition corresponding to high temperature by keeping the mice in a temperature higher than normal, 39°—41° C. Their temperature could be made to rise as high as 38°—39° C. We shall treat the effect of the temperature of the surroundings on metabolism later.

EXPERIMENTS TO PRODUCE AMYLOID

We first made a preliminary experiment in which sodium caseinate was injected between 10—25 times during 12—41 days into mice kept at room temperature (Table 2). 2 out of 8 mice died, one after 8 and the other after 14 injections.

The results are illustrated in Tables 3—7. The investigations shown in Table 7 were carried out on rats. In the other cases mice were used as test animals. The tables also show the time during which the injections took place, the number of the injections given and the ratios of the plasma protein. Depending on the amount of amyloid discovered, marks +, ++, and +++ were used.

Summing up the results we note that there are no marked

TABLE 2

PRELIMINARY INVESTIGATIONS FOR PRODUCING AMYLOID IN MICE AT ORDINARY ROOM TEMPERATURE AFTER INJECTION OF NA CASEINATE. APPEARANCE OF AMYLOID IN THE LIVER, SPLEEN, AND KIDNEYS, AND RATIOS OF PLASMA PROTEIN

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
1	10	9/2—23/2 48	—	+	—			
2	15	9/2— 2/3 48	—	+	—	2.1	4.8	30.4
3	18	9/2— 6/3 48	+	+	—			
4	20	9/2—10/3 48	—	—	—	2.1	4.3	32.8
5	22	9/2—13/3 48	+	+	—	2.5	3.5	41.6
6	25	9/2—20/3 48	—	+	+	2.2	2.4	45.4

TABLE 3 a

TEST SERIES I. APPEARANCE OF AMYLOID IN MICE KEPT IN SUPER-NORMAL TEMPERATURE (39—41° C) AFTER INJECTIONS OF NA CASEINATE

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
7	3	19/3—26/3 48	∓	∓	+			
10	6	19/3—28/3 48	+	—	—	2.1	4.2	33.3
11	6	19/3—28/3 48	+	+	+			
12	7	19/3—31/3 48	++	++	+	2.1	2.7	43.7

TABLE 3 b

TEST SERIES I. APPEARANCE OF AMYLOID IN MICE AT ORDINARY ROOM TEMPERATURE AFTER INJECTIONS OF NA CASEINATE

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
8	4	19/3—24/3 48	+	+	+			
9	6	19/3—28/3 48	—	+	—	2.5	3.7	40.9
13	7	19/3—31/3 48	—	—	—	2.4	3.9	38.0

TABLE 4 a

TEST SERIES II. APPEARANCE OF AMYLOID IN MICE KEPT AT SUPER-NORMAL TEMPERATURE (39—41° C)

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
14	4	26/3— 3/4 48	—	+	—	2.3	3.9	37.0
16	6	26/3— 5/4 48	+	+	—			
17	6	26/3— 5/4 48	—	+	—			
18	6	26/3— 5/4 48	—	+	+	2.3	3.7	38.3
20	7	26/3— 9/4 48	++	+	+			
21	8	26/3—10/4 48	+	+	+			
22	8	26/3—10/4 48	++	++	+	1.8	2.5	41.9

TABLE 4 b

TEST SERIES II. APPEARANCE OF AMYLOID IN MICE AT ORDINARY ROOM TEMPERATURE AFTER INJECTIONS OF NA CASEINATE

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
15	4	26/3— 3/4 48	—	—	—	2.1	3.6	36.8
19	6	26/3— 5/4 48	—	—	—	2.5	3.7	40.3
23	8	26/3—10/4 48	—	—	—	2.6	3.5	42.6
24	13	26/3—17/4 48	+	—	—	2.3	1.9	54.8
25	13	26/3—17/4 48	+	+	—	2.1	2.7	43.7
26	15	26/3—20/4 48	—	+	+	1.8	2.5	41.9
27	15	26/3—20/4 48	—	+	—	1.5	2.2	40.5

TABLE 5 a

TEST SERIES III. APPEARANCE OF AMYLOID IN MICE AT SUPER-NORMAL TEMPERATURE (39—41° C) AFTER INJECTIONS OF NA CASEINATE

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
28	6	14/4—24/4 48	+	—	—	2.7	2.9	48.2
29	7	14/4—25/4 48	+	+	—			
30	7	14/4—25/4 48	+	+	—			
31	7	14/4—25/4 48	+	—	—			
32	7	14/4—25/4 48	++	—	+			
33	7	14/4—25/4 48	+++	—	+			
34	7	14/4—25/4 48	++	+	—	3.1	2.9	51.6
36	7	14/4—25/4 48	+	+	—	2.2	2.2	50.0

TABLE 5 b

TEST SERIES III. APPEARANCE OF AMYLOID IN MICE AT ORDINARY ROOM TEMPERATURE AFTER INJECTIONS OF NA CASEINATE

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
35	7	14/4—26/4 48	—	—	—	3.1	2.9	51.6
37	11	14/4— 2/5 48	—	+	—	2.6	3.0	46.4
38	11	14/4— 2/5 48	—	—	—	2.8	3.0	48.3
39	11	14/4— 2/5 48	—	±	—	1.9	2.2	45.2

TABLE 6 a

TEST SERIES IV. APPEARANCE OF AMYLOID IN MICE AT SUPER-NORMAL TEMPERATURE (39—41° C). NA CASEINATE INJECTED EVERY DAY

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
41	2	16/10—18/10 48	—	—	—	2.2	2.6	45.8
42	3	16/10—19/10 48	—	—	—	2.1	3.6	36.8
44	4	16/10—20/10 48	+	—	—	2.1	2.7	43.7
46	5	16/10—21/10 48	—	+	—	2.7	2.8	49.0
49	6	16/10—22/10 48	+	+	—	2.3	2.3	50.0
50	7	16/10—23/10 48	—	+	+	3.1	2.9	51.6
52	8	16/10—24/10 48	+	+	—	3.1	3.6	46.9
55	9	16/10—25/10 48	+	—	+	3.4	3.1	52.3
57	10	16/10—26/10 48	+	+	+	2.5	2.3	52.1
59	11	16/10—27/10 48	+	—	+	2.3	1.9	54.8

TABLE 6 b

TEST SERIES IV. APPEARANCE OF AMYLOID IN MICE AT ORDINARY ROOM TEMPERATURE. NA CASEINATE HAS BEEN INJECTED EVERY DAY

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
40	2	16/10—18/10 48	—	—	—	2.0	2.4	45.4
43	3	16/10—19/10 48	—	—	—	2.1	2.3	47.7
45	4	16/10—20/10/48	—	—	—	2.0	2.4	45.4
47	5	16/10—21/10 48	—	—	—	1.9	2.5	43.2
48	6	16/10—22/10 48	—	+	—	2.2	2.2	50.0
51	7	16/10—23/10 48	—	—	—	2.5	3.7	40.3
53	8	16/10—24/10 48	—	—	—	1.9	2.4	48.8
54	9	16/10—25/10 48	—	+	—	2.8	1.6	63.6
56	10	16/10—26/10 48	—	—	—	2.1	2.6	45.7
58	11	16/10—27/10 48	—	+	+	3.4	3.1	52.3

differences between Tables 3 a and 3 b. Yet mouse No. 12, which had been exposed to super-normal temperature showed distinct formation of amyloid in the liver, spleen, and kidneys. Mouse No. 13, on the other hand, which had been at ordinary room temperature, exhibited no such development. Considerable differences occur in Tables 4 a

TABLE 7

INVESTIGATIONS FOR PRODUCING AMYLOID IN RATS AT ORDINARY ROOM TEMPERATURE AFTER INJECTION OF NA CASEINATE

No.	Number of Injections	Time	Liver	Spleen	Kidney	Albumin %	Globulin %	Relative albumin %
R_1	7	13/3—30/3 48	+	+	+	2.0	3.9	33.9
R_2	7	»	+	+	—	2.5	3.2	43.9
R_3	20	13/3—19/4 48	—	—	+	2.1	2.9	42.0
R_4	20	»	—	—	—	1.9	2.5	43.1
R_5	32	13/3—12/5 48	—	—	—	2.9	2.9	50.0
R_6	32	»	—	—	—	2.7	2.8	49.0
R_7	32	»	—	—	—	3.1	3.6	46.9
R_8	33	13/3—14/5 48	+	+	+	3.1	2.7	53.4
R_9	33	»	—	+	+	3.2	3.6	52.9

TABLE 8

RATIO OF PROTEIN AND GLOBULIN IN CASES (MICE AND RATS) BELONGING TO THE CONTROL MATERIAL

No.	Mice			Rats		
	Albumin %	Globulin %	Relative albumin %	Albumin %	Globulin %	Relative albumin %
C_1	3.4	2.8	54.8	3.1	2.9	51.6
C_2	4.2	2.5	62.7	3.1	2.6	54.4
C_3	4.4	2.7	62.0	3.4	2.8	54.8
C_4	3.5	2.6	57.4	3.4	3.0	53.1
C_5	3.4	2.8	54.8	3.3	2.9	53.2
C_6	3.1	1.7	64.5			
C_7	3.0	2.3	56.6			
C_8	3.5	2.4	59.3			
C_9	3.1	2.2	58.5			
C_{10}	4.6	2.5	64.8			
Mean	3.6	2.4	59.5	3.3	2.8	53.4

and 4 b. In mice which had been in a raised temperature amyloid could be noted regularly as early as after the 4th injection. At room temperature liver amyloid was not produced in the test animals until after the 13th injection. When comparing these tables we observe, in addition, that in the mice which had been exposed to heat the amount of amyloid increased as the number of injections increased,

and it was present, in the liver, the spleen, and the kidneys. In mice at room temperature, on the other hand, amyloid was not found contemporaneously in all these organs. When comparing Tables 5 a and 5 b we note similar results. In this experiment the temperature in the terrarium was raised during the experiment with the consequence that most of the mice died after the 7th injection. In specimens taken immediately after death amyloid formation was disclosed in every test animal. During the same time (Table 5 b, No. 35) no amyloid was found in any mouse which had been kept at room temperature, but was present already after the 11th injection of caseinate. Tables 6 a and 6 b show clearly that the formation of amyloid was more intensive in the mice which had been exposed to heat and that it occurred earlier than in the mice which had been kept at ordinary room temperature. Amyloid was regularly discovered in the former after the 4th injection, when 5 days had elapsed, and in the latter after the 6th injection. The greater the number of caseinate injections given, the more distinct was the amyloid formation. Thus the mice which had been exposed to heat showed amyloid in the liver, spleen, and kidneys.

Table 7 illustrates the amyloid experiments made on rats. It is interesting that amyloid should have been produced in only 4 out of the 9 test animals within about 2 months. This seems be an indication of how predisposition may vary considerably in these animals.

No marked divergencies were noted as regards *the appearance of amyloid in the different organs.* Yet amyloid did not always seem to develop quite as easily in the kidneys as in the liver and the spleen. After a sufficient number of sodium caseinate injections amyloid was in most cases discovered in the kidneys as well. Amyloidosis of the liver and the spleen generally seemed to occur contemporaneously. Some of the examined mice also had amyloid formation in the pancreas. In amyloid cases the iodine test revealed macroscopically a typical mahogany brown colour formation in the liver, spleen, and kidneys.

After the injections of sodium caseinate had been started we could generally notice at autopsy an enlargement of the parenchymatous organs of the abdominal cavity, especially of the spleen. These organs also seemed to contain more blood than the same organs in the control animals.

The time within which amyloidosis developed varied considerably. The shortest time was 4 days, after which amyloid was found after two injections in the liver of a mouse which had been exposed to heat (Table 6 a, mouse No. 44). One mouse, however, showed some formation suspiciously like amyloid both in the liver and spleen as early as after 2 days, after two injections. In most cases no amyloid was disclosed until after 7—14 days, i.e., after the 4th—8th injection of sodium caseinate.

This is clearly revealed in Tables 6 a and 6 b, showing how sodium caseinate injections were given every day to find out the day the amyloid took to showing, and the daily results from the second injection. In the mouse which had been exposed to heat amyloidosis of the liver was noticed after 4 days, after four injections, and in the test animal which had been at ordinary room temperature after 6 days, after the 6th injection. Some mice, on the other hand, showed no amyloid even after the lapse of almost three weeks, as we can see from Table 5 b.

On some test animals we also made examinations for *urine albumin*. No albumin could be discovered with certainty, though in some cases some typical opalescence pointing to albumin was present. These tests were especially difficult, because there was so little urine.

Changes characteristic of the *alarm reaction* were revealed at autopsy in most of the animals injected with sodium caseinate. They were especially distinct in the parenchymatous organs of the abdominal cavity. The adrenals were reddish and enlarged, as were the liver and the kidneys, and especially the spleen. They were noticeably softer and flabbier and contained more blood than the corresponding organs in the control animals. Several of the animals had a number of petechiae in the mesentery, as well as in the peritoneum.

Histological Investigation

After the first sodium caseinate injections foci of liver cell necrosis were noted here and there in the *liver*, in connection with which inflamed cells both with lobed and round nuclei occurred. Amyloid does not yet appear. In cases which had been under treatment for a longer time there was variation, besides in the foci of necrosis, in the size of the nuclei of the liver cells, evidently due to regenera-

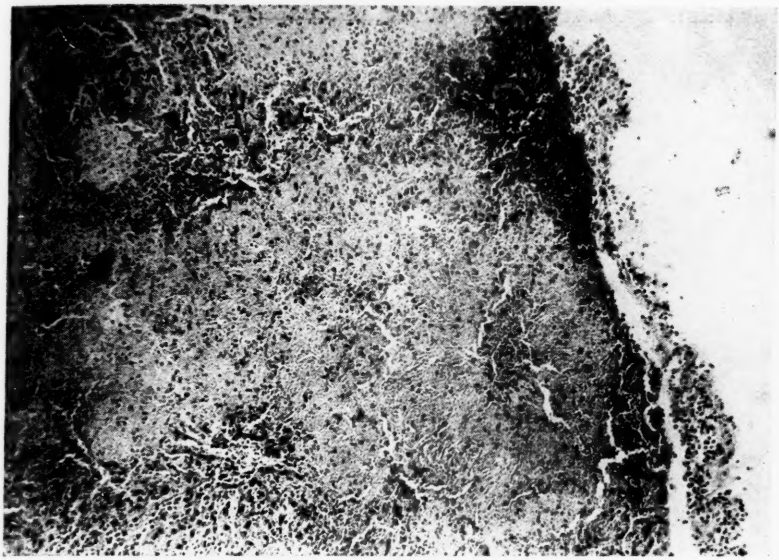

Fig. 1 a. — Test animal No. 30. Liver tissue in which amyloid areas are lighter in colour. The subject was given 7 injections of Na caseinate on 12 days at super-normal temperature. Cf. Fig. 1 b. 85 ×.

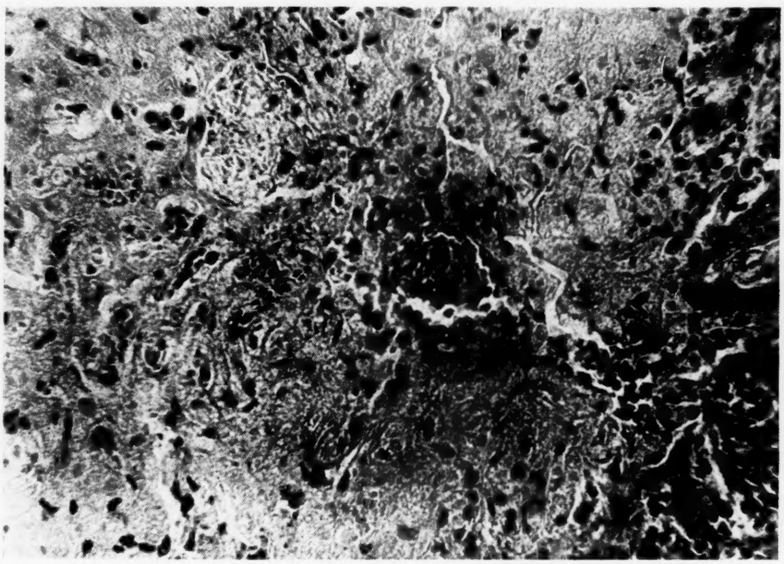

Fig. 1 b. — On necrotic area homogenous substance giving amyloid reaction (+). Increased number of cells around amyloid areas. Considerable number of inflammatory cells. 300 ×.

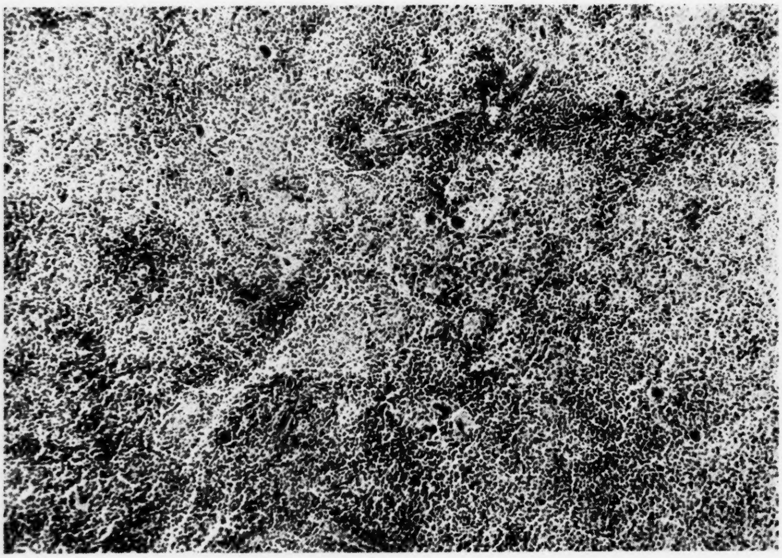

Fig. 2 a. — Test animal No. 12. Splenic tissue with amyloid on the light areas. Kongo red stain. The subject had been given 7 injections of Na caseinate on 13 days at super-normal temperature (39—41° C). Cf. Fig. 2 b. 85 ×.

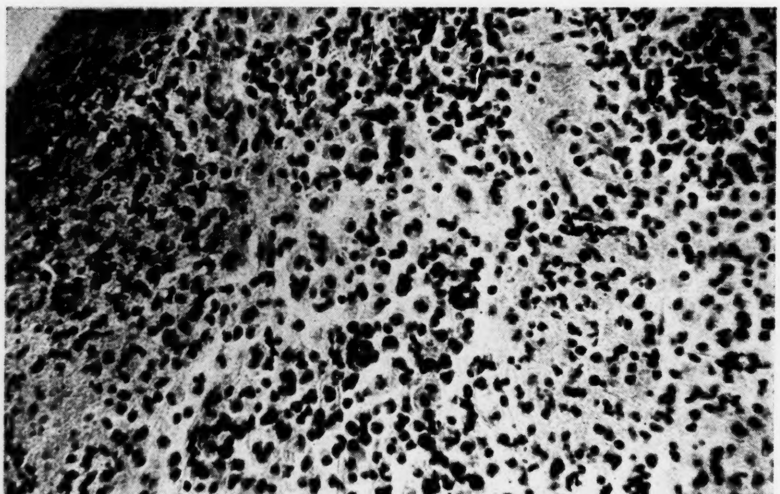

Fig. 2 b. — Cellular tissue belonging to reticulo-endothelial system is hypertrophic and increased. Lymphatic tissue is atrophied, reactional centres poorly developed. A fair amount of granulocytes in pulp. In centre of tissue homogenous substance giving amyloid reaction. 300 ×.

Fig. 3 a. — Test animal No. 20. Nephric tissue with homogenous substance giving amyloid reaction. 7 injections of Na caseinate given on 14 days at supernormal temperature. Cf. Fig. 3 b. 85 ×.

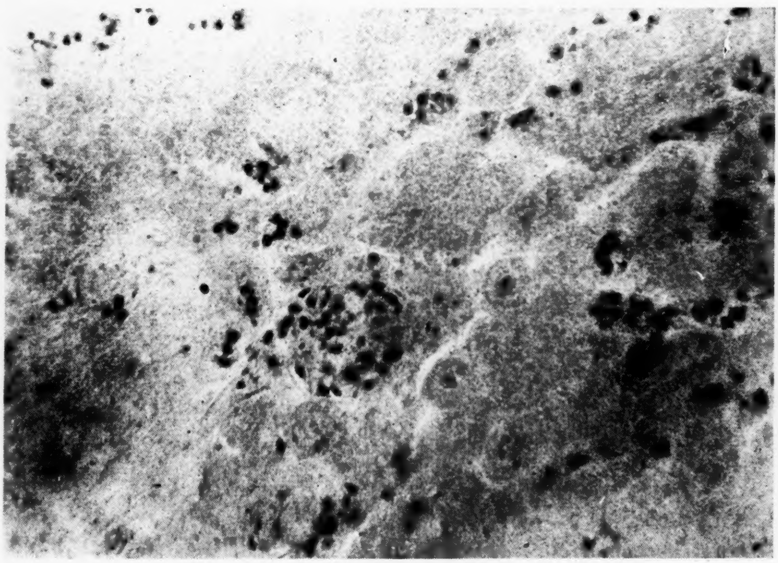

Fig. 3 b. — Nephric tissue. Nephrosis and necrosis on the area of tubules. Nephrotic areas have been necrotized. Amyloid in glomeruli and elsewhere. Test animal was given 3 injections of Na caseinate on 6 days at supernormal temperature. 300 ×.

tive changes. Amyloid occurred chiefly in the area of the necrotic foci and to some extent more diffusely elsewhere as well. In the periportal necrotic area slight increase of the connective tissue was noted in animals which had been subjected to more prolonged experiments. The sinusoids were rich in cells, partly because of the inflamed cells and partly due to hypertrophy of the Kupfer star cells. Most of them showed fatty degeneration and hypertrophy of the reticulo-endothelial cells (cf. Fig. 1 a and 1 b).

In the *spleen* evident hyperaemia and hypertrophy of the cells belonging to the reticulo-endothelial system could be seen after the first injections of sodium caseinate, and also slight atrophy of the lymphatic tissue. Yet no amyloid occurred. After continued injections of sodium caseinate an evidently increasing hypertrophy and also hyperplasia of the cells belonging to the reticulo-endothelial system was apparent. At the same time the lymphatic tissue atrophied and in some cases it could be seen to disappear almost entirely. Then necrotic areas appeared, containing a homogenous substance which gave amyloid reactions. A certain number of granulocytes occurred. The amyloid seemed to develop primarily in the reticulo-endothelial tissue. The necrosis developed first and afterwards the amyloid, which gradually seemed to destroy the reticulo-endothelial tissue (cf. Fig. 2 a and 2 b).

In the *kidneys*, after repeated sodium caseinate injections, there was increasing nephrosis and degeneration. Necrosis occurred, besides, especially in the area of the tubules and in the most superficial parts of the cortex. In the necrotic areas amyloid was present in some cases. The amyloid was most frequent in the glomeruli, but also elsewhere in the tissue. In severe nephrosis necrosis seemed to develop first and after that the precipitation of the amyloid evidently took place in these areas (cf. Fig. 3 a and 3 b).

BLOOD INVESTIGATIONS

Ratio between Albumin and Globulin

As we said earlier, hyperglobulinaemia and amyloidosis are usually coincidental. In our investigations we also determined the plasma proteins in order to follow changes in their quantity and above all if any divergence could be noted in the ratio between protein and globulin in cases of exposure to heat compared with

cases kept at room temperature. Infectious diseases in themselves give rise to hyperglobulinaemia. The question is now whether the increasing metabolism brought about by the warm surroundings may also possibly have this effect — without the bacteria having their usual effect upon the organism.

To estimate the results 10 normal mice and 5 rats were examined as a controls. These results appear in Table 8.

The investigations on mice after the injections of sodium caseinate are seen in Tables 2, 3 a and 3 b — 6 a and 6 b, and those for rats in Table 7. When comparing the values in these Tables with the protein and globulin ratio in the control cases a distinct difference may be noted.

Table 8 shows that in the control cases the relative protein percentage for 10 mice varies from 54.8 to 64.8 per cent and is on an average 59.5 per cent. In rats, correspondingly, the relative protein percentage ranges between 51.6 and 54.8 per cent, averaging 53.4 per cent. When comparing the results obtained in the experiments with these values we find that in the rats injected with sodium caseinate the relative protein percentage is as a rule distinctly lower. The limit values for the protein percentage are 30.4 per cent (Table 2, mouse No. 2) and 63.6 per cent (Table 6 b, mouse No. 54).

After injection of caseinate no marked difference could be seen in the protein and globulin ratio of mice kept in ordinary room temperature and of these kept at a super-normal temperature. On the other hand it seemed evident that in many cases the hyperglobulinaemia of the mice was more noticeable in the investigations made at the beginning of the experiment than in the test animals examined during the later stages. In many instances, indeed, the hyperglobulinaemia almost seemed to disappear after the development of amyloidosis. This appeared to be the case if the formation of amyloid was abundant, which tendency is especially apparent in Tables 6 a and 6 b.

This tendency was even more obvious in the investigations made on rats, as we see from Table 7. In five control animals the limit values of the relative protein percentage were 51.6 per cent and 54.8 per cent, with an average of 53.4 per cent. In the first animal of the series (Table 7) the relative protein percentage was 33.9 and in rat No. 8 had the highest value 53.4 per cent. The average was

47.2 per cent. In the last-mentioned test animal amyloid degeneration was present both in the spleen and the kidney.

The described experiments showed that when sodium caseinate solution was injected into mice and rats, hyperglobulinaemia resulted in comparison with the control animals. The condition seemed to decrease as amyloidosis developed and when the injections were continued.

White Blood Cell Count

As noted earlier, some investigators have found in cases of amyloid in the bone marrow of man, lively increase of the cells indicating leukopoiesis. To study these changes we compared the white blood cell counts of 36 of the mice and 9 rats with those of the control animals. Slide preparations were made in the usual manner. Special note was also taken of the appearance of plasma cells.

The white cell counts of 10 mice and 5 rats belonging to the control series appear in Table 9. We see that neutrophil leukocytes averaged 14.0 per cent, cells with lobed nuclei 13.5 per cent, monocytes 1.5 per cent, and lymphocytes 85.5 per cent. No early stages of white cells nor eosinophil nor basophil cells were present. Leukocytes with rod-shaped nuclei were encountered in one mice. No plasma cells were found in any of the mice, but in one of the five rats there were some.

Tables 10 and 11 give the figures for the white cells and the plasma cells in mice and rats, which had been given regular injections of sodium caseinate. When comparing the values obtained for the 10 rats with the control values (Table 9) we note certain differences. *The amount of neutrophil leukocytes generally seemed to be higher than the average amount in control animals.* The highest value was 48.0 per cent and the lowest 2.0 per cent, the corresponding figures for the control animals being 25.0 and 9.0 cent. In nine cases the number of neutrophil leukocytes was greater than the highest figure for the control animals. Yet even lower figures occurred incomparison with the control cases, such as 2.0 per cent and 4.0 per cent (mice No. 19 and 56). The number of neutrophil leukocytes often seemed to be higher when compared with the control animals, in those test animals in which amyloid was discovered.

A decrease in *lymphocytes* mostly coincided with an increase in

TABLE 9

WHITE AND PLASMA CELLS OF BLOOD (200 CELLS) IN CONTROL MICE AND RATS

	Mice									Rats								
No.	Neutrophils	Early Stages	Rod Forms	Segmented Neutrophils	Eosinophils	Basophils	Monocytes	Lymphocytes	Plasma Cells	Neutrophils	Early Stages	Rod Forms	Segmented Neutrophils	Eosinophils	Basophils	Monocytes	Lymphocytes	Plasma Cells
C_1	19.0	—	—	19.0	—	—	1.5	90.0	—	20.0	—	1.5	18.5	0.5	—	3.5	75.5	0.5
C_2	25.0	—	—	25.0	—	—	1.0	79.0	—	27.0	—	—	27.0	—	—	1.5	72.5	—
C_3	16.0	—	—	16.0	—	—	0.5	83.5	—	25.0	—	—	25.0	—	—	2.0	80.0	—
C_4	6.5	—	—	6.5	—	—	1.5	92.0	—	35.5	—	0.5	35.0	0.5	—	2.5	61.5	—
C_5	19.5	—	0.5	19.0	—	—	4.0	76.5	—	23.0	—	—	23.0	—	—	1.0	78.0	—
C_6	19.0	—	—	19.0	—	—	2.5	78.5	—									
C_7	10.0	—	—	10.0	—	—	—	90.0	—									
C_8	24.0	—	—	24.0	—	—	3.0	73.0	—									
C_9	9.0	—	—	9.0	—	—	—	91.0	—									
C_{10}	8.0	—	—	8.0	—	—	1.0	91.0	—									
Mean	14.0	—		13.5	—	—	1.5	85.5	—	26.1	—		25.7		—	2.1	73.5	

TABLE 10

AMOUNTS OF DIFFERENT WHITE AND PLASMA CELLS OF BLOOD OF MICE AFTER INJECTIONS OF NA CASEINATE

THE TABLE ALSO SHOWS POSSIBLE OCCURRENCE OF AMYLOID AND WHETHER TEST ANIMAL HAD BEEN IN SUPER-NORMAL TEMPERATURE (T_1) OR AT ORDINARY ROOM TEMPERATURE (T_2)

No.	Neutro-phils	Early Stages	Rod Forms	Segmented Neutrophils	Eosino-philes	Baso-phils	Mono-cytes	Lympho-cytes	Plasma Cells	Appearance of Amyloid	
										T_1	T_2
5	14.0	—	1.0	13.0	—	—	8.0	78.0	—		+
6	20.0	—	—	20.0	—	—	6.0	74.0	—		+
9	16.0	—	—	16.0	—	—	6.0	78.0	—		+
10	8.0	—	—	8.0	—	—	7.0	21.0	—	+	
13	22.0	—	6.0	16.0	1.0	—	2.0	75.0	—		—
14	33.0	—	3.0	30.0	—	—	7.0	60.0	—	+	
15	13.0	—	—	13.0	—	—	7.0	80.0	—		—
18	10.0	—	—	10.0	—	—	6.0	84.0	—	+	
19	2.0	—	—	2.0	—	—	6.0	92.0	—		—
22	12.0	—	1.0	11.0	—	—	5.0	83.0	—	+	
23	11.0	—	—	11.0	—	—	1.0	88.0	—		—
24	13.0	—	—	13.0	—	—	13.0	74.0	—		+
25	44.0	—	2.0	42.0	—	—	4.0	52.0	—		+
26	45.0	—	4.0	41.0	—	—	7.0	49.0	—		+
27	22.0	—	1.0	21.0	—	—	13.0	65.0	—		+
35	16.0	—	2.0	14.0	—	—	3.0	81.0	—		—
36	30.0	—	4.0	26.0	—	—	12.0	58.0	—	+	
37	30.0	—	3.0	27.0	—	—	10.0	59.0	—		+
38	36.0	—	2.0	34.0	2.0	—	2.0	60.0	—		—
39	10.0	—	—	10.0	—	—	—	90.0	—		±
40	25.0	—	—	25.0	—	—	3.5	71;0	0.5		—
41	22.0	—	—	22.0	—	—	2.0	76.0	—	—	
42	23.5	—	0.5	23.0	—	—	5.0	71.5	—		—
43	29.0	—	0.5	28.5	—	—	2.0	69.0	—		—
45	13.0	—	—	13.0	—	—	—	87.0	—		—
47	18.0	—	—	18.0	—	—	0.5	81.5	—		—
48	27;5	—	—	27.5	—	—	3.5	68.5	0.5		+
49	22.0	—	—	22.0	—	—	—	78.0	—	+	
50	14.5	—	—	14.5	—	—	9.0	67.0	0.5	+	
52	19.0	—	—	19.0	—	—	8.0	72.5	0.5	+	
54	13.0	—	—	13.0	—	—	1.0	86.0	—		+
55	20.0	—	—	20.0	—	—	4.0	76.0	—	+	
56	4.0	—	—	4.0	—	—	—	96.0	—		—
57	48.0	—	—	48.0	—	—	1.0	51.0	—	+	
58	10.0	—	—	10.0	—	—	—	90.0	—		+
59	23.0	—	—	23.0	—	—	5.0	72.0	—	+	

TABLE 11

NUMBERS OF DIFFERENT WHITE CELLS OF BLOOD (200 CELLS) AND OCCURRENCE OF PLASMA CELLS IN RATS AFTER INJECTIONS OF NA CASEINATE

No.	Neutrophils	Early Stages	Rod Forms	Segmented Neutrophils	Eosinophils	Basophils	Monocytes	Lymphocytes	Plasma Cells	Appearance of Amyloid
R_1	59.0	—	—	59.0	—	—	1.0	40.0	—	+
R_2	48.5	—	—	48.5	—	—	1.5	29.0	—	+
R_3	24.0	—	1.0	23.0	—	—	10.0	66.0	—	—
R_4	17.0	—	—	17.0	1.0	—	2.0	80.0	—	—
R_5	21.0	—	0.5	20.5	—	—	8.0	71.0	0.5	—
R_6	25.0	—	—	25.0	—	—	7.0	68.0	1.0	—
R_7	12.0	—	—	12.0	1.0	—	2.0	85.0	—	—
R_8	18.5	—	1.0	17.5	—	—	3.5	78.0	—	+
R_9	37.5	—	1.5	36.0	0.5	—	0.5	62.0	—	+

the number of the neutrophil leukocytes. This seems to be the case in mice No. 14, 25, 26, 36, 37, 38, 48, and 57.

Early stages of leukocytes did not occur in any of the animals. *Cells with rod-shaped nuclei*, on the other hand, were present in 13 test animals. The limit values were 0.5 and 6.0 per cent. In the control series only one had these cells. The number of cells with lobed nuclei was not outstandingly different from that of neutrophil cells.

In comparison with the control cases, the amount of *monocytes* was greater in most of the test animals, the highest value being 13 per cent (Nos. 24 and 27). Yet there appeared to be no distinct correlation between this figure and the amount of neutrophil leukocytes or lymphocytes.

Nothing special was noted in the occurrence of *plasma cells*. They were present only in four test animals (Nos. 40, 48, 50, and 52), 0.5 per cent in each. Three of them exhibited amyloidosis.

The examinations made on rats are surveyed in Table 11. In them a tendency similar to that in the mice could be observed in the ratio of the white cells of the blood.

The above results show that in mice which had been given an injection of 5 per cent sodium caseinate an increase of neutrophil leukocytes occurred when compared with the control animals. A simultaneous decrease in lymphocytes was noted in several of the test ani-

mals. It was most obvious in those cases where amyloid degeneration was found. The amount of the monocytes also seemed to have increased in many cases compared with the control animals. No increase was noted in the number of the plasma cells.

IV

DISCUSSION

As could be seen at the examination of the results, amyloid was discovered in the mice after injections of sodium caseinate in the liver, spleen, and kidneys. *Environmentalt emperature hitgher han the normal body temperature of the test animals clearly seemed to contribute to the development of the amyloid.*

What the actual mechanism of the origin of the amyloid is and how the effect of the temperature is to be explained are questions not easy to answer. Is the injection of a foreign protein followed by some specific or non-specific reaction of the organism which then causes the formation of amyloid? In clinical investigations on the electrolyte metabolism a rise in temperature was found to have furthered such conditions as hypoproteinaemia, hypochloraemia, and acidosis (SCHALLOCK, PERÄSALO). A rise in temperature in itself obviously intensifies metabolism. In these cases several other factors such as the endocrine glands are influential. The thyroid gland and the adrenals, especially, are known to play an important part in the regulation of the body temperature.

It is generally believed that the development of amyloid is a result of *the antigen-antibody reaction* of the organism (LETTERER and LOESCHKE). During our investigations we were able to observe an enlargement of the liver and especially of the spleen as early as the second and the third day after the injection of sodium caseinate solution. They were full of blood, and the reticular cells, besides, were enlarged. After continued injections the splenic reticular cells kept growing bigger compared with the corresponding cells of the control animals. The same observation was made by LOESCHKE. This might be explained as follows: The cells belonging to the reticulo-endothelial system phagocytize and dissolve

the foreign protein. At the same time antibodies are formed, and their formation is increased by the continued injections. In rats, LOESCHKE observed the formation of antibodies after caseinate injections, not only against the casein but also against leukocyte protein. In fact, he regarded amyloid as a leukocyte precipitation, a protein formed by antigens and antibodies. It must then always be presupposed that the organism is made sensitive to leukocyte protein and that the blood contains a great surplus of antigen.

The experiments on mice and rats showed hyperglobulinaemia after injections of sodium caseinate, which seemed to decrease with the increasing number of injections and when amyloid had begun to form. This seems to bear out LOESCHKE's theory.

When the factor causing a disease, or some kind of irritation, continues to act for a sufficiently long time, the capacity of the organism to form antibodies is exhausted and a surplus of antigen results in the blood. As we know, the antibodies exist mainly in the globulins. We might thus assume that a treated test animal which has a high content of serum globulin but has not developed amyloid may be capable of forming a great number of antibodies. On the other hand, an animal whose serum globulin concentration remains low is an indifferent producer of antibodies and will consequently develop amyloidosis. Amyloidosis would thus only occur when the body is either a poor manufacturer of antibodies in general or displays, in a diseased condition, a temporary decline in immunity. The rabbit is a good and the mouse a poor producer of precipitin (LOESCHKE). The former therefore seldom contracts amyloidosis, the latter often. The food has also been found to play a certain part in the formation of amyloid. It is characteristic that such a typical »alkaline» animal as the guinea pig never develops amyloidosis while the »acid» animal the mouse does.

In the white cell count of the blood evident increase of neutrophil leukocytes was observed as compared with the control animals, and a simultaneous decrease in lymphocytes. In estimating these results one should naturally remember the variation in the number of the blood cells. Marked as it is in man, it is no doubt still more marked in animals. Consequently we cannot speak of any »normal values.» A limited comparison is possible, however, with the use of the control material.

A number of reactions pointed to *the general-adaptation-syn-*

drome. As we said, these symptoms did not attract our notice until the final phases of the work. We thus did not pay sufficient attention to this reaction. The different stages of the alarm reaction were not observed. However, since studies are still being carried on, this point will also be investigated.

Typical symptoms indicative of the alarm reaction were the enlargement of the adrenals, spleen, liver, and kidneys. They were full of blood, reddish and flabby. Haematoma occurred in the mesentery in several cases. In the liver fatty degeneration was noted histologically, as was necrosis and hypertrophy of the endothelium of the sinusoids and of the Kupfer star cells. Hypertrophy of the cells belonging to the reticulo-endothelial system occurred in the spleen, as well as atrophy or complete disappearance of the lymphatic tissue. Both nephrosis and necrosis were discovered in the kidneys. The changes in the blood picture also, such as leukocytosis and lymphopaenia, are typical reactions of the adaptation syndrome. Slight monocytosis was noted in some cases, in addition.

The alarm reaction is a kind of physiological mechanism capable of increasing the power of resistance of the organism to the irritant to which the body is exposed. The adrenals are of special importance in this change of the resistance. According to SELYE every stimulus detrimental to the organism makes (in some way so far unexplained) the hypophysis secrete a corticotrophic hormone, which in its turn stimulates the adrenals to an increased formation of various hormones and especially corticoid hormone. By the intervention of the lymphatic organs and the lymphocytes the hormones stimulate the development of antibodies.

On this basis we could also explain both the obvious hypertrophy of the cells belonging to the reticulo-endothelial system after the injections of sodium caseinate, which was noticeable during our investigations in the liver, spleen, and kidneys, and also, the reduction in the lymphocyte count and the hyperglobulinaemia. Sodium caseinate is evidently a powerful substance eliciting an intense reaction in the organism of the test animal. The increased hypophysis-adrenal function would lead to atrophy and destruction of the lymphatic tissue, which was clearly noticeable in the spleen, especially in the mice. The corticoid hormone secreted by the adrenals would in these cases be of especial importance. In contrast to

the atrophy of the lymphatic tissue we noted evident hypertrophy and hyperplasia of the cells belonging to the reticulo-endothelial system in the liver, spleen, and kidneys of mice. The immediate consequence is hyperglobulinaemia and also evident increase in the formation of antibodies.

The formation of amyloid in the liver, spleen and kidneys was best seen in the necrotic areas, which mainly consisted of cell groups belonging to the reticulo-endothelial system. This was especially obvious in the spleen, where the amyloid formed to replace the necrotized cells of the reticulo-endothelial system and as it were destroyed this tissue. At the same time the lymphatic tissue almost disappeared. Considerable variation could be seen in the kidneys. Yet it seemed in most cases that first necrosis and then amyloid developed on the basis of severe nephrosis. The necrosis which set in was in the first place obviously a result of the toxic effect of the sodium caseinate.

The fact that in our animal experiments no increase in the neutrophil cells nor any lymphopaenia occurred in all the cases can be explained. We know that in the beginning of the alarm reaction — in the shock phase — leukopaenia is usually present. There were indications of this condition in some mice, in which the number of the neutrophil leukocytes was only 2—4 per cent. The amyloid in itself did not seem to be of any decisive importance for the increase of the neutrophil leukocytes and the decrease in the lymphocyte count, for these changes were also noticeable in some test animals which did not develop amyloid.

We could observe during the investigations that an environmental temperature which was higher than the normal body temperature of the mice seemed to promote the formation of amyloid. Temperature has a marked influence upon metabolism. Animals at a low stage of development are thus much more dependent upon external circumstances — especially upon temperature — than higher animals. At a low temperature the reactions of the organism are slower. Rise in the temperature of the surroundings raises the temperature of the body even to exceed 39° C (Ott et al., 1948). Along with an increase in temperature metabolism intensifies. Mac Connel and Yaglolou (1924) made experiments on metabolism on man, with this in mind, at different »effective temperatures». At temperatures between 75—83° F the values for metabolism were lowest, but

still somewhat increased. At temperatures over 90° F metabolism showed a very marked increase. Similar investigations have also been carried out by numerous other investigators (BENEDICT, C. G., BENEDICT, F. G., and DUBOIS 1925).

The respiratory exchange of gases can be regarded as a relative indicator and measure of the total metabolism. PETERS, BULGER. EISEMAN and LEE, and KOST, MEYERS and SCHMITZ noted slight decrease of the CO_2 amount in feverish subjects in general. The investigations made by these authorities also support the view that the effect of a rise in body temperature is comparable with an increase in the rate of breathing caused by environmental heat, a decline in the CO_2 tension, and the set-in of the CO_2 deficiency. Yet this is usually slight in feverish conditions (PETERS and VAN SLYKE, 1937).

When considering the reason for the increased metabolism caused by heat we have to bear in mind *the part the endocrine glands admittedly play in the regulation of the temperature of the organism.* The thyroid gland and the adrenals, especially, have an important role in the heat regulation of the body. *Cannon* (1919) observed that exposure to cold caused an increase in the rate of the denervated heart. It has also been discovered that the serum taken from an animal exposed to cold increases the metabolism of a second animal into which it is injected. If the first animal has been thyroidectomized, no effect upon the metabolism of the second is observed (BEST and TAYLOR, 1945). The adrenal secretion exerts a calorigenic effect which is immediate and of short duration. Its liberation follows short periods of cold. The experiments of UOTILA (1939) point to the pituitary as being responsible for the reaction to cold, for hypophysectomy usually causes thyroid atrophy even when the animals have been exposed to low temperature for long periods. High body temperature and infection are said to cause a reduction in the supply of stored adrenalin. A stimulation of the hypothalmus has also been found to elicit discharge of adrenalin. It may be mentioned, in addition, that the temperature tends to be subnormal in suprarenalectomized or thyroidectomized animals.

The bone marrow of the test animals which had developed amyloidosis showed changes indicative of erythropoiesia and leukopoiesia and especially an increase of cells belonging to the reticulo-endothelial

system, and of plasma cells. Thus the observations made on them in the present study are in agreement with the research performed on man in cases of amyloidosis (ROHR, RANSTRÖM).

From the observations made it seems evident that the effect exerted by super-normal temperature in promoting the formation of amyloid is, in the first place, a consequence of increased metabolism. Metabolism, on the other hand, is influenced by the hypophysis — adrenal mechanism as well as certain other factors. The other endocrine glands may also be of some importance in these cases.

It may be suggested that the formation of amyloid itself is a kind of alarm reaction of the organism or a reaction belonging to the total adaptation syndrome. The type of the lesion, the resistance of the organism and the hypophysis-adrenal mechanism would then be the most essential factors. The atrophy of the lymphatic tissue and the increase of the cells belonging to the reticulo-endothelial system would then be attributable, mainly, to the hyperfunction of the adrenals. The immediate consequence of the increase of the reticulo-endothelial cells would be hyperglobulinaemia and an intensified formation of antibodies. The precipitation of amyloid would take place along with an increase of hyperglobulinaemia and a contemporaneous decline in the power of resistance.

SUMMARY

A survey is made of opinions in the literature on amyloid degeneration and of experimental research on amyloid and the significance of the bone marrow in the development of blood protein.

The object of the work was to investigate the formation of amyloid experimentally at temperatures exceeding the normal. 59 mice and 9 rats were used as test animals. The control material consisted of 10 mice and 5 rats. Some blood studies were also carried out, determining the ratio between protein and globulin, the bone marrow was investigated, and an analysis of the white cells of the blood was made.

To *produce amyloid* the writers used a 5 per cent sodium caseinate solution, which was injected into the test animals subcutaneously every other day. In mice the amyloid developed much more easily than rats though considerable individual variation was noticed even in them. The rats were injected with casein in the course of 2—6 weeks. Amyloid was discovered in only four of the rats, in two of them after about a fortnight.

We first tried to produce a rise in temperature with a suspension made of various strains of Bact. fae. alcaligenes »Pyrisan» and sulphurated »Sulfurol». These substances, however, could not be proved to have the desired effect. We did not find the use of other substances advisable and consequently tried to produce a corresponding state by keeping the laboratory animals in a temperature above the normal.

When comparing the results obtained for mice in ordinary room temperature with those arrived at with mice kept in a temperature higher than normal (39°—41° C), it could be noted that in the latter the amyloid generally developed more rapidly. This was more obvious when the number of injections given to the test-animals was larger. At ordinary room temperature the amyloid developed

at the earliest on the 7th day after six injections and in mice exposed to super-normal temperature on the 4th day after 3 injections.

Simultaneously with the formation of amyloid the writers could notice hyperglobulinaemia as compared with the control animals. This hyperglobulinemia was, however, often slighter than in the test animals, which had no amyloid.

In the blood picture evident increase of neutrophil leukocytes was observed as compared with the control animals, and a decrease in the lymphocyte count. In cases with amyloid, increase of the cells belonging to the reticulo-endothelial system was apparent.

Besides amyloidosis, changes pointing to an alarm reaction could be noticed not only in the blood but also in the liver, spleen, kidneys, and adrenals.

The mechanism of the amyloid formation was finally treated, especially as a reaction associated with the general-adaptation-syndrome.

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